At the outset, the Examiner is respectfully thanked for the courteous interview held on December 15, 1998, with James Robl, Ph.D., an inventor of this application, the Examiner and the undersigned. During the interview, the outstanding rejections were discussed in detail. In particular, the Examiner was advised as to Applicants' intent to cancel the product claims and, further, was advised that the claims would be amended consistent with proposed independent claims presented at the interview. It is noted for the record that Applicants have indeed cancelled the product claims herein, and have rewritten the claims consistent with the interview.

Also, the outstanding §112, first paragraph, enablement rejection was discussed in great detail. All of the points raised by the Examiner were respectfully traversed based on the §132 Declaration, which was informally presented to the Examiner at the interview. It was explained that the subject invention comprises a pioneering discovery, i.e., that somatic cells or cells committed to a somatic cell lineage may be used as nuclear transfer donors for cloning desired non-human mammals by nuclear transfer techniques. It was indicated that this was a surprising discovery as it was contravened by previous accepted dogma in the art. Essentially, prior to the present invention, it was thought that once a cell becomes differentiated that it loses its ability to be a suitable donor cell during nuclear transfer. More specifically, as explained in Dr. Robl's Declaration, it was widely thought by researchers working in the area of cloning prior to the present invention that,

once a cell becomes committed to a particular somatic cell lineage, its nucleus irreversibly loses its ability to become "reprogrammed", i.e., to support full-term development when used as a nuclear donor for nuclear transfer.

1. Also, the particular §112 issues raised by the Examiner were discussed. It was explained and substantiated by scientific literature that the efficacy of the subject cloning technique is not dependent upon the particular somatic cell, nor does it depend on the particular *in vitro* maturation technique or the specific culture media used to maintain the nuclear transfer embryo prior to introduction into a female surrogate. Moreover, it was argued, and also substantiated by supporting documentation, that the subject cloning method is generically applicable to non-human mammals. The Examiner was advised that, subsequent to the filing of this application, numerous groups have successfully cloned bovines using fetal and adult cells employing the techniques of the invention and, moreover, sheep, goat, and mice have also been successfully cloned using somatic cells as the donor cell for nuclear transfer. It was also stated and substantiated by supporting documentation that such cloning has been successfully conducted with numerous somatic cell types and using adult and fetal cells. In response to such arguments, the Examiner indicated that she would consider this evidence in detail

when it was formally presented in a subsequent Response and when the Declaration was formally submitted.

Also, as discussed above, the Examiner reviewed proposed independent claims. Based on the Examiner's review, the proposed claims were revised to take into account her suggested changes. Based on the following, it is anticipated that this Response, together with the §132 Declaration and references submitted herewith, should place this application in condition for allowance.

Turning now to the Office Action, Applicants acknowledge the double patenting rejection of Claims 1-34 and 55-77 based on copending U.S. Serial No. 888,283. The Examiner is respectfully requested to hold this rejection is abeyance until this application is otherwise allowable. Moreover, the Examiner is respectfully requested that, if this is the only outstanding issue, to allow this application and to subsequently apply the double patenting rejection, if necessary, in the remaining pending application.

Similarly, Claims 1-34 and 55-77 stand rejected under the doctrine of obviousness-type double patenting as being unpatentable over Claims 1-35 and 47-77 of U.S. Serial No. 888,057. The Examiner is similarly respectfully requested to hold this rejection is abeyance until this application is otherwise allowable and, if this is the only outstanding issue, to allow this application and maintain the double patenting rejection, if necessary, in the remaining pending application.

Claims 1-16, 23, 24, 28-34, 55, 56, 58, 60, 62, 63, 65, 67, 69, 70, 78 and 79 were rejected under 35 U.S.C. §101 on the basis the claims were directed to non-statutory subject matter. This rejection should be moot as the current claims are now restricted to cloning non-human mammals. The basis of the §101 rejection was that the prior claims read on cloning of humans. This obviously was not Applicants' intent and the claims have been amended in order to make this expressly clear. Based on the foregoing, withdrawal of the §101 rejection is respectfully requested.

Claims 33 and 34 were rejected under 35 U.S.C. §112, first paragraph, as assertedly containing subject matter not described in the application so as to enable one skilled in the art to make and/or use the invention. This rejection should also be moot as the current claims no longer contain the phrase "non transformed", which assertedly did not find appropriate support in the disclosure. However, Applicants respectfully maintain that this phrase finds implicit support in the Examples because it is clear that the subject cells which are used as nuclear transfer donors are not transformed. However, in any event, this rejection is now moot as this language does not appear in the current claims.

Claims 1-34 and 55-79 also were rejected under 35 U.S.C. §112, first paragraph, on the basis that the disclosure assertedly only enabled methods for cloning a bovine comprising inserting a fibroblast or the nucleus of the fibroblast isolated from a 45 day pregnancy bovine fetus into the perivitelline space of a bovine oocyte matured *in vitro* to

metaphase II, followed by fusion of the cell or nucleus with the oocyte to form an NT unit with activation being effected by incubating NT units at 26 to 27 hours post-maturation of the oocyte using ionomycin and DMAP under the conditions recited at page 5, first paragraph, of the most recent Office Action, with the resulted nuclear transfer embryo then being cultured using the CR1aa media containing mouse fibroblast feeder cells with the resultant cultured nuclear transfer embryo then being transferred in to a surrogate host bovine for development into a fetus and/or offspring and progeny. Based on the following, the position of the Examiner is respectfully traversed.

It is anticipated that this rejection will be overcome upon consideration of the §132 Declaration by Dr. James Robl which accompanies this Reply. Therein, Applicants address all of the issues raised by the Examiner and provide supporting evidence which substantiates that none of these particular steps are critical to the efficacy of the subject invention. Rather, as discussed above, the present invention involves the generic discovery that cells committed to a somatic cell lineage or somatic cells or nuclei derived therefrom which are capable of division may be used as nuclear transfer donors during nuclear transplantation, and give rise to cloned non-human mammalian embryos, fetuses, and offspring.

Turning to the specific limitations urged to be critical, the Office Action first indicates that the efficacy of the subject invention is dependent upon the age of the donor

somatic cell, and also on the particular somatic cell type. However, this is not correct. To the contrary, as substantiated by subsequent reports, somatic cells of various different ages may be used as successfully as nuclear donors for nuclear transfer. All that is essential is that the somatic cell be capable of division. While the Examiner is correct to the extent that adult cells are less efficient for cloning than are fetal cells, this does not substantiate a position that adult cells cannot be successfully used for cloning. In fact, as disclosed in the subject application, and as has been subsequently reported in the literature, adult cells also can be used for nuclear transfer donors and give rise to viable offspring. For example, as discussed during the recent interview, numerous Japanese groups have recently successfully cloned a number of cows using various different adult cells as the donor cells for nuclear transfer. This is discussed in paragraph 17 of Dr. Robl's Declaration. Moreover, the efficacy of the cloning method is not dependent upon the particular somatic cell type either. As discussed in paragraph 17 of the Robl Declaration, non-fibroblast cells have been successfully used for cloning purposes, e.g., oviduct cells, muscle cells and cumulus cells have been successfully used for cloning non-human mammals.

Instead, all that is important to the invention is that the somatic cells used as the donor be capable of division. As explained in paragraph 18 of the Declaration, cells meeting the appropriate conditions, i.e., which are capable of division, are found in both

fetal and adults. Moreover, while the length of the cell cycle and the life-span of cells shortens with the age of the donor, it is emphasized that cell populations obtained from adult cells comprise cells capable of active division, albeit in lesser numbers than in younger animals. Thus, based on the foregoing, the efficacy of the subject invention does not depend upon the particular age of the donor cell, nor does it depend on a particular somatic type. To reiterate, the only essential feature of the claimed method is that the donor cell be a non-quiescent somatic cell, i.e., a somatic cell capable of division. The importance of division to the efficacy of the invention is discussed, in particular, in the context of fibroblast cells. For example, at page 20 of the subject application, it is reported that fibroblast cells are an ideal cell type because they can be readily obtained from developing fetuses and adult animals in large quantities, and can be easily propagated *in vitro* with a rapid doubling time. Therefore, the importance of active division to the donor cell finds clear support in the disclosure.

The Office Action further alleges that *in vitro* maturation and the particular *in vitro* maturation procedure were critical to the invention. However, this also is not the case. While the Examiner is correct in the fact that *in vitro* matured oocytes are exemplified in the actual working Examples, this is not essential to the efficacy of the subject cloning methods. In fact, cloning has been successfully conducted using *in vivo* matured oocytes. In particular, as discussed in Dr. Robl's Declaration, the inventors recently cloned a

transgenic bovine using *in vivo* matured oocytes. As Dr. Robl explains in his Declaration, *in vitro* matured oocytes were exemplified merely because of supply and cost concerns. They were utilized because immature oocytes (rather than *in vivo* matured oocytes) were available in plentiful numbers, e.g., from slaughterhouse suppliers. However, as substantiated by the fact that a cloned animal has been obtained using *in vivo* matured oocytes, it is clear that the specifics of *in vitro* maturation is not essential to the subject cloning methods.

Nor is the particular means for *in vitro* activation essential to the invention. Again, as properly described by the Examiner, the application exemplifies a particular *in vitro* activation technique which comprises activating the NT unit by incubating 26 to 27 post *in vitro* maturation by incubation in a media comprising specific amounts of ionomycin and DMAP, followed by culturing the NT units in a CR1aa-2mM DMAP medium for four to five hours. However, this particular *in vitro* activation procedure is also not essential to the invention. Indeed, as discussed in detail at pages 14 through 18 of the Declaration by Dr. Robl, those skilled in the art were aware of many different methods for effecting *in vitro* activation at the time the present application was filed.

In general, these methods involve the elevation of intracellular calcium in the egg, which occurs normally *in vivo* upon contacting the egg with sperm. However, this may be effected using many different methods, e.g., using ethanol, electrical shock, cooling,

calcium-free medium, various anesthetics, and a variety of other stimuli. The Declaration summarizes, in paragraph 22, various literature references that teach alternative *in vitro* activation methods, which would be expected to be suitable for use in cloning methods according to the invention. Moreover, for the Examiner's convenience, an overview of different activation procedures is summarized in tabular form at page 17 of the Declaration. Based on the discussion therein, it is clear that there are a number of different activation procedures which can be used for oocyte activation that are conducted by various laboratories around the world, and the use of ionomycin/DMAP was chosen more a matter of convenience than because it was better than other protocols. Thus, contrary to the Office Action, it is not critical to the success of the subject cloning protocols. Therefore, based on the foregoing, withdrawal of this basis of the rejection is also respectfully requested.

Another basis for the §112 enablement rejection was the Examiner's position that the particular culture medium used to maintain the nuclear transfer embryos was essential to the cloning protocol. However, this also is not correct. Again, as substantiated by the Declaration of Dr. Robl, specifically at paragraph 23, the particular exemplified (CR1aa) culture medium is not critical to the efficacy of the invention. In fact, there are many different media that could be used interchangeably for growing bovine embryos in culture. For example, suitable media for culture of bovine embryos include, by way of

example, simple media, complex media, co-culture systems containing cumulus cells, BRL cells, or fibroblast cells, and completely defined media. Moreover, Dr. Robl further advises in his Declaration that, even in his lab, different students are utilizing different media and have had success with the development of embryos. Again, for the Examiner's convenience, a brief overview of different culture media which may be successfully used to maintain bovine embryos in culture is summarized in tabular form at page 17 of the Robl Declaration.

Finally, Applicants respectfully maintain that efficacy of the subject cloning protocol is not limited to bovines. To the contrary, and as discussed at length during the interview, Applicants have made a generic discovery, namely that non-human mammals may be successfully cloned by nuclear transfer using as the nuclear transfer donor a somatic cell or nucleus derived therefrom which is capable of division. The fact that Applicants have made a generic discovery is substantiated by the results of numerous groups who have successfully cloning animals using such cells subsequent to the filing of this application. The fact that the subject cloning protocol is generic to different non-human mammals is substantiated by paragraphs 24 and 25 of Dr. Robl's Declaration. Therein, Dr. Robl advises that somatic cells have been successfully used to clone sheep, mice, bovines (using fetal and adult cells) and goats. Also, the generic nature of the subject discovery is supported by the cross-species nuclear transplantation work also

being conducted by the present inventors. As has been recently well reported in the Press, the present inventors conducted an experiment wherein they produced a nuclear transfer embryo by the insertion of an adult human somatic cell into a enucleated bovine oocyte. After activation, they obtained what appeared to be a blastocyst stage embryo. This further substantiates the fact that adult differentiated cells of different mammalian species can be successfully "reprogrammed" notwithstanding the previous dogma that only very early non-differentiated cells could be used as nuclear transfer donor cells or nuclei.

Therefore, based on the foregoing, it is believed that Applicants have persuasively rebutted all of the bases for the enablement rejection. In particular, Applicants have demonstrated and substantiated, based on the §132 Declaration of Dr. Robl, that the efficacy of the subject invention does not rely upon the age of the donor cell, nor does it depend upon a particular differentiated cell type, or the specific *in vitro* activation procedure or the specific culture medium used to maintain the nuclear transfer embryos. Finally, Applicants have demonstrated that the subject cloning methodology is generic in nature, i.e., that it may be successfully used for cloning different, non-human mammals, including mice, goats, cows, and sheep. As discussed at the interview, the fact that mice have been successfully cloned using somatic cells especially demonstrates the generic nature of the invention given the significant differences in embryonic development between mice and bovines. Also, Applicants respectfully submit that the

present inventors are entitled to broad claims given the pioneering nature of the subject invention. Indeed, as discussed in the Declaration, the subject invention flies in the face of previous dogma in the area of cloning, namely the mistaken idea that only very early non-differentiated cells could be successfully used as donor cells or nuclei for nuclear transfer. Therefore, based on the foregoing, withdrawal of the §112 enablement rejection, which was previously applied to Claims 1-34 and 55-79, is respectfully requested.

Claims 33-34 also were rejected under 35 U.S.C. §112, second paragraph, as assertedly being indefinite in the recitation "transformed". This issue should be moot as this language no longer appears in any of the new claims.

Claims 17-19, 25-27, 71-73, 75 and 77 were rejected under 35 U.S.C. §102(b) as assertedly being anticipated by USP 5,057,420. This rejection is moot as there are no claims now directed to fetuses, offspring, or progeny. As discussed above, these claims have been cancelled without prejudice or disclaimer, and these claims may be subsequently represented in a divisional application.

Claims 20-22, 57, 59, 61, 64, 66, 68, 74 and 76 also were rejected under 35 U.S.C. §102(b) as assertedly being anticipated by Hyttinen (1994), *Bio/Technology*, 12:606-608. This rejection should also be moot as there are no current claims directed to transgenic chimeric and transgenic/chimeric fetuses. Again, these claims were cancelled without prejudice in the interest of expediting allowance of this application.

Claim 29 was rejected under 35 U.S.C. §102(b) as assertedly being anticipated by Sims et al. This rejection is also moot as there are no claims currently directed to a CICM cell line. This subject matter has also been cancelled in order to expedite prosecution.

Claims 17-19, 20-22, 25-27, 29, 57, 59, 61, 64, 66, 68 and 71-77 were also rejected based on Hyttinen and Sims. This rejection is also moot as this subject matter is no longer claimed.

Claims 33, 34, 78 and 79 were rejected under 35 U.S.C. §102(b) as being anticipated by Kono et al. These claims were directed to differentiated cells and human cells made by the claimed process, wherein the cells are not transformed. This rejection is also moot as the claims directed to such differentiated cells and human cells have been cancelled in order to expedite prosecution.

Finally, Claim 31 was rejected under 35 U.S.C. §103(a) as assertedly being unpatentable over Sims et al (1993), *Proced. Natl. Acad. Sci.*, 90:6143-6147, in view of Lovell-Badge et al, *Cold Spring Harbor Symp. Quant. Biol.*, Vol. 50, 707-711. This claim was directed to a transgenic CICM cell line. This rejection is also moot as Applicants no longer claim this cell line. This subject matter has also been cancelled in order to expedite prosecution. The Examiner is also respectfully advised that this subject matter may be resubmitted in a divisional application.

Based on the foregoing, it is believed that the foregoing amendments and remarks, coupled with the §132 Declaration by Dr. Robl and attachments thereto, should place this case in condition for allowance. A Notice to that effect is respectfully solicited. **Moreover, given the great importance of this application, the Examiner is respectfully requested to contact the undersigned after receipt and consideration of this Reply, assuming that any issues remain outstanding. In particular, the Examiner is respectfully requested to contact the undersigned in connection with any proposed Examiner's amendment that would place this case in condition for allowance. Also, Applicants would be amenable to a subsequent telephonic or personal interview with the Examiner, with or without an inventor if necessary, in order to resolve any outstanding issues.**

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: _____

Robin L. Teskin
Registration No. 35,030

Post Office Box 1404
Alexandria, VA 22313-1404
(703) 836-6620

December 22, 1998